REMARKS

1. Status of the Claims

Claims 10 and 15-18 have been amended. Claims 19-23 have been cancelled. New claims 24-31 have been added.

Claim 10 has been amended to correct a typographical error. Claim 15 has been amended to be in independent form and has included the limitations of claim 1. Claims 16-18 have been amended to include the limitation "virally safe". Claim 18 has been amended to depend on claim 15 and to include the limitations of claim 14. No new matter has been added.

Support for new claims 24-31 can be found in the original claims.

2. Status of the Specification

In the pending Office Action, the disclosure is objected to since the Examiner considers that a brief description of the drawings title section is missing.

A Brief Description of the Drawings section has been added to the specification.

Support for the elements disclosed in the added Brief Description of the Drawings section can be found in the specification of the application as filed, and in particular in example 3, at page 16, lines 10-18, in example 4, at page 17, lines 7-10, in example 5, at page 18, lines 1-5, in example 6, at page 19, lines 1-7 and 9-11, in example 7, from page 19, line 21 to page 20, line 1, and at page 20, lines 10-13.

It is believed that the objection should now be withdrawn.

3. Claim rejection under 35 U.S.C. §103

Claims 15 to 18 have been rejected as being obvious under 35 U.S.C. § 103 over WO 92/04367, in view of EP 0 570 916 and *Winge* (US 6,399,357 B1). This rejection is respectfully traversed.

The Examiner considers in particular that WO 92/04367 discloses a first method for purification of albumin by the use of dyes immobilized on a column of ion-exchange resins, and suggests the use of an alternative method using Cibacron Blue dyes. The Examiner thus purports that, in view of these teachings, one of ordinary skill in the art would be motivated to look for supplementary steps which could assure the intended product (albumin) to achieve the required purity. Further, the Examiner considers that EP 0 570 916 teaches a method of purifying human serum albumin and purports that this application describes filtration as a routine operation to improve the purity of a protein. The Examiner finally purports that one of ordinary skill in the art would thus be motivated to employ nanofiltration, since *Winge* is purported to disclose the purification of albumin using a nanofilter.

Applicant, for the following reasons, submits that the prior art references do not teach or suggest the present invention, whether considered alone or in combination.

Applicant submits that the albumin product of the present invention is distinct from and has advantageous properties as compared to the products in the cited references. The product of the invention is importantly:

1) purified via nanofiltration instead of via heat treatment to make the product virus free.
2) free of contamination by reagents used in prior art methods.

3.1. Distinctions Over the Primary Reference WO 92/04367

Applicant respectfully submits that WO 92/04367 actually discloses a method for purifying proteins consisting in the combination of an ion exchanger process with the use of a high salt/caprylate concentration to disrupt the dye-protein binding, such as to allow the efficient separation of the dye from the desired protein (see WO 92/04367 at page 2, lines 12-16).

As disclosed at page 3, lines 9-11, the process of WO 92/04367 is

> "*particularly well suited to removing synthetic textile dye compounds of the sort which have been disclosed in the literature for purifying proteins.*"

This document indicates in particular that, depending on the protein to be purified, the ion exchanger could either be selected among cation-exchangers such as S and CM Fast Flow, or anion-exchangers such as DEAE Fast Flow and Q-Fast flow (see page 6, lines 8-12).

Further, WO 92/04367 discloses that elution of serum albumin is obtained through the use of a disrupting material, comprising a mixture of a salt and of a compound capable to disrupt hydrophobic interactions, such as fatty acid salts:

> "*The disrupting material may be a single compound or a mixture. Preferably, it comprises a mixture of a salt (preferably sodium chloride or potassium chloride) and a compound to disrupt hydrophobic interactions between the protein and the protein-binding compound, for example a (preferably non-ionic) detergent, an organic solvent or, preferably, a fatty acid.(...)*
> *The fatty acid is preferably octanoic acid but other fatty acids (preferably C6-C10 and preferably saturated) may be used. The fatty acid will usually be present in the form of its salt, for example the sodium salt.*" (see WO 92/04367, page 9, lines 9-15 and 18-22).

Finally, Examples 1 to 4 of WO 92/04367 disclose a process for purifying human serum albumin from a fermentation medium, consisting in mixing said fermentation medium with a dye, then adding a disrupter of hydrophobic interactions (sodium octanoate or caprylate), and passing this solution through an anion exchanger (Dowex-1 resin). As disclosed in table 1, page 12, the

process disclosed allowed contaminants such as "dye + spacer" or "dye intermediate" to be removed until a maximum efficiency of 97% and 96% respectively.

WO 92/04367 fails to disclose or even suggest a method for purifying albumin comprising additional purification steps, such as for example a filtration step, and more specifically, nowhere discloses a nanofiltration step. Further, WO 92/04367 fails to disclose any method comprising a virus inactivation step.

Applicant therefore submits that the albumin composition resulting from the purification process of WO 92/04367 is not virally safe and contains contaminants which are not present in the product of the present invention.

The albumin solution of the present invention is virally safe and is devoid from reagents used in classical purifications methods, as described in the present application:

> "*Therefore, the object of the present invention is to provide a solution corresponding to a good compromise between two criteria, the retention efficiency of viruses and/or other macromolecules likely to induce diseases or side effects in patients, and the recovery yield of albumin*" (see the present application, page 6, lines 3-8), and
>
> "*The aqueous albumin solutions are solutions free from any reagent employed during various classical steps of albumin manufacture or purification, such as e.g. polyethyleneglycol (PEG), ethanol, organic salts (sodium caprylate, etc.) and inorganic salts.*" (see the present application, page 7, lines 10-15).

Applicant therefore submits that one of ordinary skill in the art would have had no reasonable expectation of success to prepare the albumin solution of the present invention in view of the teachings of WO 92/04367.

3.2. Even a Combination of WO 92/04367 with EP 0 570 916 Fails to Teach the Present Invention

Applicant submits that the teachings of EP 0 570 916 do not rescue the deficiencies of WO 92/04367.

EP 0 570 916 actually discloses a process for producing a recombinant human serum albumin comprising the steps of (see EP 0, 570 916, from page 2, line 50 to page 3, line 11):

(1) ultrafiltrating a culture supernatant on two membranes of decreasing molecular weight limits (100,000 to 500,000 and 1,000 to 50,000, respectively),
(2) heat-treating the filtrate at 50 to 70°C for 30 minutes to 5 hours,
(3) acid-treating the heated sample at pH 3 to 5,
(4) ultrafiltrating the acid-treated sample on a membrane with a molecular weight limit of 100,000 to 500,000,
(5) separating the serum albumin through chromatography on a cation exchanger,
(6) separating the serum albumin through hydrophobic chromatography,
(7) separating the serum albumin through chromatography on an anion exchanger, and, possibly,
(8) separating the serum albumin through chromatography on a chelate resin.

EP 0 570 916 inevitably comprises a heat-treatment, preferably conducted in presence of a stabilizing agent:

> "*(ii) Heat treatment*
> *The concentrated solution obtained in the above step (i) is subjected to heat treatment at 50 to 70°C for approximately 30 minutes to 5 hours, preferably at 60°C for approximately 1 to 3 hours.*
> *Preferably, the heating is conducted in the presence of a stabilizing agent. Preferred examples of the stabilizer include acetyltryptophan and an organic carboxylic acid having 6 to 18 carbon atoms, or a salt thereof. The stabilizers may be used in combination. Acetyltryptophan may be used in an amount of approximately from 1 to 100 mM. Illustrative examples of the organic carboxylic acid having 6 to 18 carbon atoms include caproic acid (6 carbon atoms), caprylic acid (8 carbon atoms), capric*

> *acid (10 carbon atoms), lauric acid (12 carbon atoms), palmitic acid (16 carbon atoms), oleic acid (18 carbon atoms) and the like. Illustrative examples of the salts include alkali metal salts such as sodium salt, potassium salt and the like, and alkaline earth metal salts, such as calcium salt and the like. The organic carboxylic acid having 6 to 18 carbon atoms or a salt thereof may be used in an amount of approximately from 1 to 100 mM.*" (see EP 0 570 916, page 5, lines 9-20).

Such a heat-treatment step is also extensively disclosed in the examples of EP 0 570 916:

> "*Next, the membrane fraction (I) was heat-treated at 60°C for 3 hours in the presence of 5mM of sodium caprylate, 10 mM cysteine and 100 mM of aminoguanidine at pH 7.5. The thus heat-treated solution was cooled down rapidly to about 15°C, adjusted to pH 4.5 and then treated with an ultrafiltration membrane having a molecular weight exclusive limit of 300,000.*" (see page 11, lines 20-23).

Further, it unambiguously appears in EP 0 570 916, that the resulting albumin solution is conditioned for pharmaceutical use, through the addition of acetyltryptophan or a salt thereof, and sodium caprylate:

> "*another object of the instant invention is to provide a pharmaceutical preparation comprising recombinant human serum albumin, acetyltryptophan or a salt thereof and sodium caprylate.*" (see EP 0 570 916, page 3, lines 15-16 and claim 11)

> "*The resulting pharmaceutical preparation consisted of 25% HAS, 0.02M acetyltryptophan sodium salt and 0.0M sodium caprylate.*" (see AP 0 570 916, page 16, example 10, lines 39-40)

The nanofiltered albumin solution of the invention differs significantly from the solutions disclosed in EP 0 570 916 in that the product of the present invention is obtained without the use of a heat-treatment step, but is rather based on the use of a nanofiltration step. The resulting solution is thus advantageously both virally safe and devoid of any contaminants that could possibly result from heat-treatment or from standard albumin purification steps (and in particular from sodium caprylate). This distinction is explained in the present application:

> "*The nanofiltered albumin solutions show a very high degree of safety in respect of particulate contaminants with a size of e.g. at least about 13 nm, e.g. viruses such as non-enveloped viruses, prions, albumin polymers (tetramers-decamers) generated during steps of albumin manufacture or during the pasteurisation at 60°C, micelle-like*

lipopolysaccharides, nucleic acids and aggregated proteins." (see the present application, from page 6, line 28 to page 7, line 4), and

"*The aqueous albumin solutions are solutions free from any reagent employed during various classical steps of albumin manufacture or purification, such as e.g. polyethyleneglycol (PEG), ethanol, organic salts (sodium caprylate, etc.) and inorganic salts.*"(see the present invention, at page 7, lines 10-15)

3.2.1. Binding Properties of the Product of the Invention are Superior to the Product of EP 0 570 916

In addition, the albumin of the invention retains its binding and transport potential of various active ingredients, since its binding sites are not blocked:

"*The safety achieved with these albumin compositions for therapeutic use makes it possible to suppress the pasteurisation step, a source of drawbacks as mentioned above, and therefore, to add usual protection stabilisers against thermal effects, which also bind on the albumin sites, thus preventing albumin from binding the relevant molecules. The albumin of these compositions according to the invention retains its binding and transport potential of various active ingredients and through this binding, reduces their toxicity or increases the bioavailability by a depot effect.*" (see the present application, at page 11, lines 5-15).

"*The invention also relates to a virally safe aqueous albumin solution obtainable by implementing the method of the invention, in which the transport and binding sites of therapeutically active ingredients are available in the albumin.*"(see the present application, at page 10, lines 20-24)

EP 0 570 916 (at page 16, lines 11-21) compares the binding of bilirubin (a pigment), warfarine (a drug) and lauric acid (a fatty acid) to the albumin resulting from the process described in EP 0 570 916. The binding properties of the prepared albumin to those compounds were analyzed by Scatchard plotting, and summarized in Figure 2. EP 0 570 916 concludes that the binding affinities of plasma albumlin and of the purified albumin are almost similar:

"*As shown in Fig. 2, binding curve of recombinant albumin was consistent with plasma albumin. The results that binding affinities of recombinant albumin with those ligands were almost similar to those of plasma albumin indicate the biological equivalency between both albumins.*"(see EP 0 570 916, at page 16, lines 19-21).

The binding properties of the albumin obtained by the process of the present invention, on the other hand, appear to be significantly higher than those of an albumin prepared by a process comprising a heat-treatment step. This interpretation is fully supported by the results disclosed in example 12 of the present application. In this example, the properties in the transport and binding of medicines of an albumin nanofiltered without any stabilizer are studied by comparing them with those of two different albumin batches pasteurised in the presence of sodium caprylate. As disclosed in tables 10 and 11 (pages 28 and 30), and summarized in table 12 (page 31), the albumin resulting from the process of the invention (noted "A'4") displays binding constants (Ka) for diazepam and warfarine which are significantly higher than those of the heat-treated albumins.

In view of these elements, Applicant submits that a person of ordinary skill in the art would have had no reasonable expectation of success to prepare the albumin solution of the present invention in view of the teachings of WO 92/04367 in view of EP 0 570 916.

3.3. The Present Invention is Distinct from Even a Combination of the Three Cited References

Finally, Applicant considers that the teachings of US 6,399,357 do not rescue the deficiencies of WO 92/04367, even in view of EP 0 570 916.

US 6,399,357 discloses a method of virus-filtering a solution containing at least one molecule, and in particular albumin, wherein the total salt content of the solution is fixed in a range of from about 0.2M up to saturation.

This document discloses more specifically the use of Viresolve filters, supplied by Millipore, for filtering an albumin solution in order to remove small viruses, such as the polio virus. US 6,399,357 also suggests the use of alternative filters "Planova" provided by Asahi:

> *"Virus filters are known in the art and are supplied by Millipore from Massachussets, USA and Asahi Chemical Industry Co., Ltd. from Japan, among others. Millipore supplies filters having two different types of membrane, depending on the size of the protein concerned. For instance, Millipore supplies, among others, Viresolve™/70 for proteins having a molecular weight, or relative molecular mass, of up to about 70,000, and Viresolve™/180 for proteins having a molecular weight of up to about 180,000. This latter filter can be used for monoclonal antibodies, for instance. Asahi Chemical Industry supplies, among other things, Planova™ 35 and Planova™ 15 filters, this latter filter being used to remove smaller viruses, such as the polio virus."*(see US 6,399,357, column 5, lines 41-54).

US 6,399,357 further explicitly indicates that the virus-filtering method described therein could be operated within a large range of parameters, and more specifically in presence of a wide variety of salt, in a concentration of from 0.2M up to saturation of the solution with the salt concerned:

> *"The present invention relates to a method of virus-filtering a solution that contains at least one macromolecule, by virtue of the total salt content of the solution lying in the range of from about 0.2M up to saturation of the solution with the salt concerned."*(see US 6,399,357, column 1, lines 16-20)

> *"The most important examples of anion which have such high salting-out effect are citrate, tartrate, sulphate, acetate and phosphate. Cations that can be used advantageously when practicing the present invention are monovalent cations, such as sodium, potassium, and ammonium, as well as divalent cations, such as calcium. Sodium chloride, potassium chloride, sodium acetate, and sodium citrate or combinations thereof are particularly preferred salts in accordance with the invention, in view of the advantages that are afforded by pharmaceutically acceptable additives."*(see US 6,399,357, column 3, lines 28-40).

Applicant respectfully submits that the filtration process as described in the examples of US 6,399,357 on the contrary covers only a very narrow range of filtration conditions. These conditions comprise a pH range of 5.5 to 7.4, a protein concentration between 0.5-10.0 A_{280} units (absent any reference for the actual value of a A_{280} unit), and a salt concentration not exceeding 1.5M.

It therefore appears that the majority of examples of US 6,399,357, except examples 3 and 22, simply measure the transport of proteins through the membrane by calculating the sieving coefficient, or protein permeability factor, which is

> "*given as P/R, where P is the concentration of protein on the permeate side (the filtration side) measured by adsorption at 280nm (A_{280}) and R is the concentration of protein on the retention side (R) measured by adsorption at 280nm (A_{280})*" (see US 6,399,357, column 6, lines 58-63).

Thus, these examples do nothing more than show that increased salt concentration under different filtration conditions increases the transport of proteins through a membrane of the Viresolve type.

It is also to be noted that the salt concentration used in the examples does not exceed 1.5M NaCl, and there is no example for supporting a total salt of the solution to be "up to saturation" of the salt concerned, such as described in the present specification or in the present claims. It would indeed unambiguously appear to the one skilled in the art that a solution containing a high protein concentration and a total salt concentration of near saturation would be difficult to filter as the proteins would precipitate out of solution, so it is unlikely that a method of viral filtration performed under these conditions would successfully separate viral particles from macromolecules. Obviously, after a protein (macromolecule) has precipitated, it would be retained by the filter membrane together with a virus in a filtration aiming at separating the macromolecule from that virus.

Applicant further points out that examples 3 and 22 of US 6,399,357 are the only examples which relate to filtering of solutions actually containing viral particles. In examples 3 and 22, the salt concentrations of solutions comprising virus particles are 0.15M (as control) and 1.0M, the pH of the solution is 7.0, and protein concentration is 0.5-1.0 A_{280} (absent any reference for determining the actual value of a A_{280} unit). In addition, it is noted that neither example 3 nor example 22 actually concerns a solution comprising albumin.

It is thus submitted that the results and conclusions obtained from the other examples (1-2 and 4-21), relating to filtering of solutions comprising macromolecules (and in particular albumin), devoid of virus particles and with a salt concentration of up to 1.5M, cannot be used to

extrapolate results concerning the efficiency of virus removal as if virus was present in these solutions.

It further appears that all examples of US 6,399,357 relate to the filtration of relatively high purity protein solutions. However, there is nothing in the specification to indicate what is meant by the term "pure" protein solutions. Example 12 of US 6,399,357 in particular explicitly discloses that the solution of albumin subjected to the filtration process was supplied by Pharmacia AB, Stockholm, Sweden (see column 12, lines 19-22), and was thus prepared through classical purification procedures possibly comprising a pasteurisation step in presence of stabilizers such as sodium caprylate.

US 6,399357 therefore contains no indication in the specification of how the removal of virus will be affected by carrying out a filtration method (which refers to any filtering process without limitation) using solutions with total salt of "up to saturation", varying any number of filtration parameters (e.g. macromolecule concentration of the solution, pH of the solution, purity of the material to be filtered, the flow rate through the membrane, the type of filter, etc.). It is indeed common knowledge of the skilled person in the art that the performance of the filtration methods, in particular the effectiveness of nanofiltration towards the anti-viral filtration and the resulting protein concentration, is influenced by various parameters, like the nature of the filtration membrane, pH, ionic strength, etc.

Attention of the Examiner is in particular drawn to the fact that Viresolve filters and Planova filters (or any other nanofilter characterized by its porosity) could not be regarded as fully equivalents since they differ from a technological point of view. It indeed appears that Viresolve filters, and particularly Viresolve 70 are composed of hydrophilic polyvinylidene fluoride (PVDF), whereas Planova filters, for instance, are composed of naturally hydrophilic cuprammonium regenerated cellulose. It results from what precedes that the filtration threshold of Viresolve filters, which is expressed in weight exclusion limit (70 kDa or 180 kDa), could actually not be converted nor compared with filters the threshold of which is expressed in

porosity. One skilled in the art, therefore, would not be able to transpose the operating conditions defined for Viresolve filters for filters characterized by their porosity, such as Planova filters.

Applicant thus respectfully submits that US 6,399,357 encompasses an extremely large number of filtration conditions in which any number of important filtration parameters may be varied to any extent, and that the invention of US 6,399,357 has not been disclosed in such a clear and complete way as to enable the skilled person in the art to carry out the alledged invention, i.e. the virus-filtering of solutions containing macromolecules, and in particular albumin.

In view of the above, Applicant submits that one of ordinary skill in the art would have had no reasonable expectation of success to prepare the albumin solution of the present invention in view of the teachings of WO 92/04367, in view of EP 0 570 916, or even in view of US 6,399,357.

Withdrawal of the corresponding rejection is thus respectfully requested.

4. Conclusion

In view of the above amendment, applicant believes the pending application is in condition for allowance.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant respectfully petitions for a three (3) month extension of time for filing a reply in connection with the present application, and the required fee of $1,110.00 is attached hereto.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Leonard R. Svensson Reg. No. 30,330 at the

telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

By _____

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